Synthesis and Biological Evaluation of the Pyrazole Class of Cyclooxygenase-2-Inhibitors†

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Abstract: Several 1,3,4-trisubstituted pyrazole derivatives were synthesized *via* condensation with the appropriate amine, sulphonamide, acid hydrazide, or benzyl thiosemicarbazide derivatives. The newly synthesized compounds were screened for a possible anti-inflammatory effect in a rat model of air-pouch carrageenan-induced inflammation. The results revealed that some of the newly synthesized compounds exhibited a significant anti-inflammatory effect in terms of reducing exudation and/or leukocytic accumulation at the site of inflammation. Thus, compared to carrageenan-induced inflammation group, compounds **3, 9, 13,** and **17** were particularly associated with significant decrease in both the volume of exudate and leukocyte accumulation while compounds **4**, **7**, **10**, **11** and **15** were associated with significant decrease in the volume of inflammatory exudate without a corresponding decrease in the number of accumulated leukocytes. Moreover, a docked pose of compound **17** was obtained and bound to cyclooxygenase active site of COX-2 using Molecular Operating Environment (MOE) module.

Keywords: 1,3,4-trisubstituted pyrazoles, synthesis, anti-inflammatory activity, carrageenan air pouch inflammation rat model, MOE module.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of pain and inflammation related to a large variety of pathologies [1]. However, their therapeutic use is often limited by common side effects, such as gastrointestinal (GI) hemorrhage and ulceration [2]. Therefore, a major challenge of the pharmaceutical industry is to develop drugs that have anti-inflammatory activities but lack the toxic side effects associated with currently used NSAIDs. A major mechanism of action of NSAIDs is lowering prostaglandin production through inhibition of cyclooxygenase (COX), a key enzyme in prostaglandin biosynthesis [3]. A possible dissociation of anti-inflammatory effects from GI toxicity is suggested by the discovery that COX exists in two isoforms [4,5]. One isoform (COX-1) is constitutive and regularly expressed, producing prostaglandins involved in the cytoprotection of the GI tract. The other isoform (COX-2) is associated with inflammatory states and is generally absent from all tissues, unless induced by inflammation mediators. Therefore, the inducible isoform COX-2 constitutes the real target for anti-inflammatory drugs. In fact, molecules, which are capable of being selective COX-2 inhibitors, without affecting the constitutive isoform (COX-1) [6], have proved to be excellent drugs in the treatment of inflammatory pathologies and, most importantly, are devoid of side effects, which are typical of earlier non-selective NSAIDS [7].

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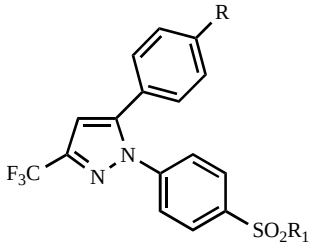
Among the already marketed COX-2 inhibitors that comprize the pyrazole nucleus, celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (Fig. **1**), which occupies a unique position as a potent and GI safe anti-inflammatory and analgesic agent. It is considered as a typical model of the 1,5-diaryl heterocyclic template that is known to inhibit selectively the COX-2 enzyme [8]. Other newly described pyrazole derivatives exemplified by SC-58125 [9] and SC-558 [10] (Fig. (**1**)) were described to exhibit selective COX-2 inhibitory action with little GI toxicity.



celecoxib	R = CH_3	$R_1 = NH_2$
SC-58125	R = F	$R_1 = CH_3$
SC-558	R = Br	$R_1 = CH_3$

Fig. (1). Chemical structures of selective COX-2 inhibitors.

Motivated by these findings, and as a continuation of the investigations in the field of pyrazole derivatives [11-14], it was designed to synthesize a novel series of pyrazole derivatives that would act as selective COX-2 inhibitors. The substitution pattern of the pyrazole ring was rationalized so as to be correlated to the diaryl heterocyclic template of compounds that has been known to act selectively as COX-2 inhibitors such as celecoxib, SC-58125, and SC-558. Using

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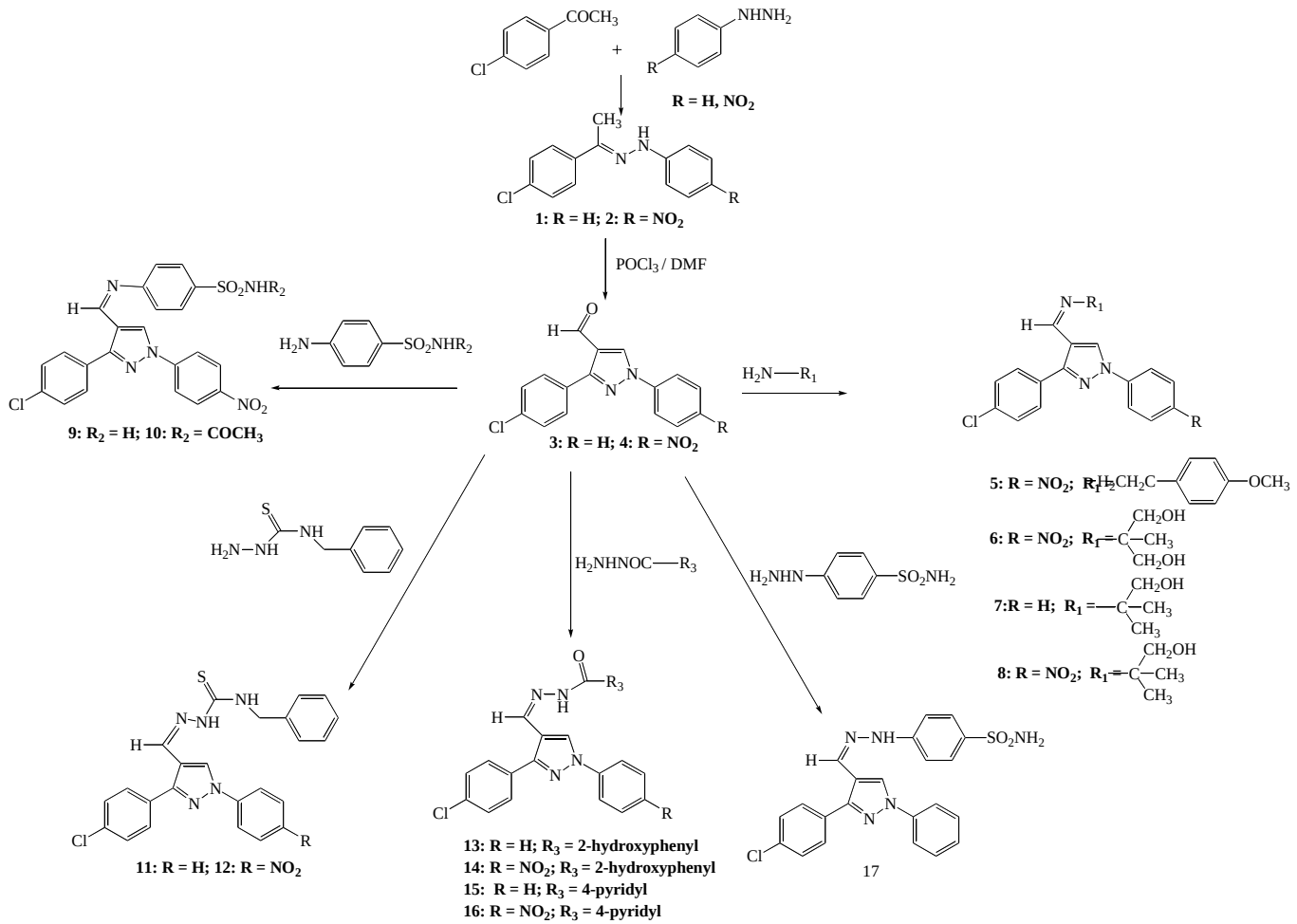

Scheme 1.

the diaryl pyrazole COX-2 template, numerous sites of modifications about the pyrazole ring system were explored. The initial change was conversion of trifluoromethyl moiety at position 3 by a p-substituted aryl moiety to increase lipid solubility and in turn bioavailability. The 4th-position was substituted with aryl moiety separated by different spacers of two, three, four or five atoms from pyrazole ring and bearing hydrogen bond donors or acceptors to increase its interaction with the corresponding receptor. These spacers could offer flexibility with regards to accommodation of benzene moiety into the large active site of COX-2 relative to COX-1. This could be in a different orientation pattern than the 1,5-diaryl pyrazole COX-2 template but could maintain COX-2 inhibitory activity.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the desired 1,3,4-trisubstituted pyrazoles **(3-17)** has been accomplished as described in Scheme **1**. The key starting materials 3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**3**) [15] and 3-(4-chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-4-carbaldehyde (**4**), were pre-pared from the reaction of 4-chloroacetophenone with phenyl hydrazine and 4-nitrophenyl hydrazine to produce the corresponding hydrazones (**1**,**2**) followed by Vilsmeier-Haack reaction [16], respectively.

Condensation of **3** and **4** with the appropriate amine, sulphonamide, benzyl thiosemicarbazide, or acid hydrazide, gave the corresponding anilide **5-10**, benzyl thiosemicarbazone **11**, **12** and hydrazone derivatives **13-17**, respectively.

The structures of the synthesized compounds were verified by IR, ^{1}H-NMR and mass spectra.

IR spectra of compound **4** showed a strong absorption band at 1700 cm^{-1} characteristic to the aldehydic carbonyl group. Disappearance of this band upon formation of compounds **5-12** is a preliminary indication for the condensation with amine and sulfonamide derivatives. IR of the new compounds **13-17** showed the appearance of strong absorption bands in the 3400-3200 cm^{-1} region, attributed to OH, NH or NH_2 stretching. Compounds **13-16** also showed a strong absorption band at 1670-1660 cm^{-1}, characteristic to the amidic carbonyl group.

^{1}H-NMR of all the synthesized compounds showed singlet signals at 8.92-9.61 attributed to CH=O or CH=N group. For compounds **13-17** an additional singlet at 9.85-10.66 was assigned to NH group. Diagnostically important signals in ^{1}H-NMR spectra of compounds **11**, **12** were two singlet signals at 8.72-8.75 and at 11.46-11.48 attributed to

Table 1. Anti-Inflammatory Effects of Tested Compounds in the Rat Air Pouch

Treatment	Leukocyte Accumulation (Cell Number x 10^{-6}/pouch)	Volume of Exudate (ml)
Vehicle-treated control	10.3 ± 8.1***	0.8 ±0.5***
Carrageenan-treated	67.8 ±17.3	4.5 ± 0.4
Indomethacin	18.6 ± 3.1***	1.3 ± 0.3***
3	28.8 ±11.8***	1.8 ± 0.3***
4	65.3 ±10.5	6.4 ± 0.1***
6	43.8 ± 7.1*	2.0 ± 0.6***
7	68.5 ± 3.4	5.3 ± 0.4**
8	47.8 ± 11.6*	3.1 ± 0.3***
9	35.6 ± 6.4**	2.7 ± 0.2***
10	60.3 ± 6.5	5.5 ± 0.2***
11	61.4 ± 11.1	5.1 ± 0.36**
12	47.1 ± 7.7*	3.5 ± 0.3**
13	34.5 ± 8.1**	2.1 ± 0.6***
14	57.8 ± 12.3	4.9 ± 0.7
15	69.3 ± 5.1	6.3 ± 0.7***
16	57.6 ± 8.5	4.1 ± 0.6
17	33.2 ± 11.5**	2.1± 0.04***

Studied groups were compared to Carrageenan-treated group and statistical significance was as follows:
*P <0.05.
** P<0.01.
*** P< 0.001.

two NH groups. In all the compounds, absorption as multiplets at δ 6.77-8.96 was assigned to aromatic protons and pyrazole C-5 H in addition to NH_2 group (D_2O exchangeable) for compounds **9** and **17**. Moreover, the mass spectra of the new compounds showed fragments corresponding to the typical chlorine isotope (^{35}Cl and ^{37}Cl) patterns.

Biological Screening

In the present study, the synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenan air pouch model [17,18]. Indomethacin was used as a reference drug. All compounds and the reference drug were tested at a dose level of 10 mg/kg and the results are presented in Table **1**. The data showed significant decrease in the volume of inflammatory exudate in the rat groups pre-treated with indomethacin (a reference drug) as well as with the compounds **3**, **4**, **6**, **8**, **9**, **10**, **13**, **15** and **17** compared to carrageenan-treated group (P<0.001). The compounds **7**, **11** and **12** have also been associated with less accumulation of inflammatory exudate compared to carrageenan-treated group but statistically to a lesser extent than the former groups (P<0.01). The present results also revealed a significant decrease in leukocytic accumulation in the inflammatory exudate in rats subjected to pre-treatment with either indomethacin or compound **3** (P<0.001), compounds **9**, **13** or **17** (P<0.01) and compounds **6**, **8** or **12** (P< 0.05); all compared to carrageenan-treated group.

Among all tested candidates, indomethacin and compound **3** were particularly associated with highly significant decrease in both the inflammatory exudate and leuckocyte accumulation (P<0.001) compared to carrageenan-treated group. It has also to be noted that compounds **4**, **7**, **10**, **11** and **15** were associated with significant decrease in the volume of inflammatory exudate without corresponding decrease in the number of accumulated leukocytes. This could be due to a drug-associated release of local leukocyte chemotactic factor(s) or the tested drug probably lacks a significant inhibitory effect on local mediators induced by tissue inflammation.

Docking of 17 in the Cyclooxygenase Active Site of COX-2

A structural similarity between **17** and the selective COX-2 inhibitors (Fig. (**1**)) encouraged us to study its binding in the cyclooxygenase active site of COX-2 using MOE module [19]. As a starting point, we used the crystal structure of SC-558 complexed with COX-2 (PDB ID: 1CX2) [10] (Fig. (**2**)).

Docking of the energy minimized conformation of **17** in the cyclooxygenase active site of COX-2 (Fig. (**3**)) showed a more or less similar pattern of binding to COX-2 as that resulting from the crystal structure of SC-558. In both cases, hydrophobic interactions were observed between trifluoromethyl group of SC-558 as well as p-chlorophenyl of **17** and Met 113, Val 116, Val 349 and Tyr 355. In addition, the p-sulphamoylphenyl moiety of SC-558 and phenyl ring of **17** sited at the same position were bound in a pocket surrounded by hydrophobic residues Leu 352, Tyr 355, Ph 518, Val 523 and the backbone of Ser 353. In case of SC-558, the sulphamoyl moiety in the 1-positiom interacted with His 90 to form a hydrogen bond, while in case of **17**, it interacted

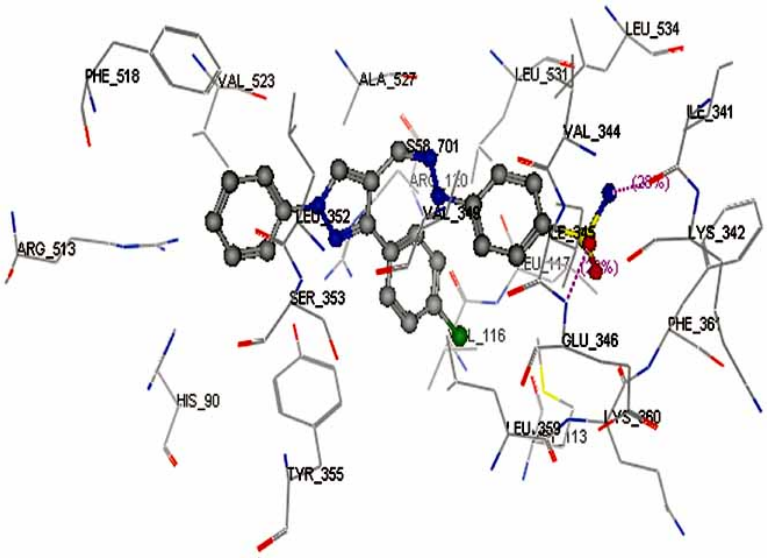

Fig. (2). Binding of SC-558 in the cyclooxygenase active site of COX-2.

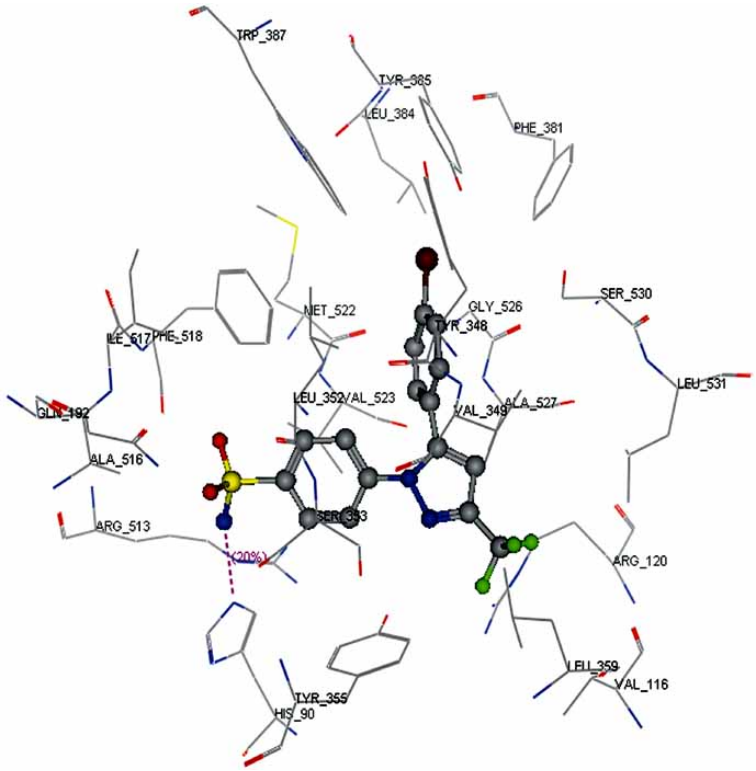

Fig. (3). 3D view from a molecular modeling study, of the minimum energy structure of the complex of 17 docked in the cyclooxygenase active site of COX-2. Pink dashed lines depict hydrogen bond interactions. Viewed using MOE module.

with Ile 341 and Glu 346 forming hydrogen bonds with these residues. It is worth-mentioning that increasing spacer between pyrazole ring and sulphonamido moiety, in order to study the effect of flexibility on the mode of binding, showed different orientation in the active site of COX-2 but maintain selective COX-2 inhibitory action and, in turn, could provide a starting point for the design of unique COX-2 inhibitors.

EXPERIMENTAL

Chemistry

Melting points were determined in open glass capillaries on Stuart melting point apparatus and are uncorrected. IR spectra ν cm^{-1} (KBr) were recorded on a Perkin-Elmer 421 Spectrophotometer. ^{1}H-NMR spectra were run on a Jeol-NMR 500 MHz Spectrophotometer, using TMS as the

internal and DMSO-d_6 as the solvent. Chemical shifts were recorded as δ (ppm). MS were obtained on a Finnigan Mass Spectrometer SSQ/7000 (70 eV). Microanalyses Elemental analyses for all the synthesized compounds were within ± 0.4% of the theoretical values. Aryl acid hydrazides were obtained from commercial sources or prepared by reported procedures [20,21].

3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1*H*-pyrazole-4-carbaldehyde (4)

Compound **2** (0.004 mole) was added to a Vilsmeier reagent prepared from DMF (10 ml) and $POCl_3$ (0.012 mole). The mixture was stirred at 60-70°C for 4 h, and then poured onto ice-H_2O mixture and neutralized with 10% $NaHCO_3$ solution. The deposited product was filtered off and crystallized from dioxane. Yield 82%; M.p. 245-247°C; Calculated for $C_{16}H_{10}ClN_3O_3$ (327.72): 58.64 %C, 3.08 %H, 12.82 %N. Found: 58.99 %C, 3.2 %H, 12.88 %N; IR, ν cm^{-1}: 1700 (C=O); ^{1}H-NMR δ: 7.41-8.25 (m, 9H, aromatic+pyrazole C-5 H), 9.31 (s, 1H, CHO).

General Procedure for Preparation of Compounds 5-16

A mixture of the appropriate aldehyde **(3,4)** (6 mmole) and an equimolar amount of the appropriate amine, sulphonamide, benzyl thiosemicarbazide, acid hydrazide in n-propanol (30 ml) and few drops of conc. sulfuric acid was refluxed for 4 h, concentrated and allowed to cool at room temperature. The precipitated product was filtered, dried and crystallized from n-propanol.

[3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-ylmethylene][2-(4-methoxyphenyl)ethyl]amine (5)

Yield 60%; M.p. >300°C; Calculated for $C_{25}H_{21}ClN_4O_3$ (460.91): 65.15 %C, 4.59 %H, 12.16 %N. Found: 65.01 %C, 4.63 %H, 11.98 %N; IR, ν cm^{-1}: 1600 (C=N); ^{1}H-NMR δ: 2.47 (m, 4H, CH_2CH_2), 3.57 (s, 3H, OCH_3), 7.51-8.32 (m, 13H, aromatic+pyrazole C-5 H), 9.17 (s, 1H, CH=N); MS, m/z (rel. abund. %): 462 (33.6, M^++2), 460 (100, M^+), 325 (13.3), 279 (31.6),136 (25.8), 76 (28.5), 63 (27.3).

2-{[3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-ylmethylene]amino}-2-methylpropane-1,3-diol (6)

Yield 58%; M.p. >300°C; Calculated for $C_{20}H_{19}ClN_4O_4$ (414.84): 57.90 %C, 4.62 %H, 13.51 %N. Found: 58.20 %C, 4.67 %H, 13.84 %N; IR, ν cm^{-1}: 3400 (OH), 1600 (C=N); ^{1}H-NMR δ: 1.07 (s, 3H, CH_3), 3.56 (d, 4H, J = 3.2 Hz, $2CH_2OH$), 7.48-8.28 (m, 9H, aromatic+pyrazole C-5 H), 9.11 (s, 1H, CH=N), 9.89 (s, 2H, 2OH).

2-{[3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]amino}-2-methylpropan-1-ol (7)

Yield 55%; M.p. 175°C; Calculated for $C_{20}H_{20}ClN_3O$ (353.85): 67.89 %C, 5.70 %H, 11.88 %N. Found: 67.51 %C, 5.93 %H, 11.44 %N; IR, ν cm^{-1}: 3420 (OH), 1610 (C=N); ^{1}H-NMR δ: 1.15 (s, 6H, $2CH_3$), 3.26 (d, 2H, J = 3.2 Hz, CH_2OH), 7.48-8.35 (m, 10H, aromatic+pyrazole C-5 H), 9.51 (s, 1H, CH=N), 9.91 (s, 1H, OH).

2-{[3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-ylmethylene]amino}-2-methylpropan-1-ol (8)

Yield 58%; M.p. > 300°C; Calculated for $C_{20}H_{19}ClN_4O_3$ (398.84): 60.23 %C, 4.80 %H, 14.05 %N. Found: 59.91 %C, 4.69 %H, 14.38 %N; IR, ν cm^{-1}: 3410 (OH), 1600 (C=N); ^{1}H-NMR δ: 1.14 (s, 6H, $2CH_3$), 3.22 (d, 2H, J = 3.2 Hz, CH_2OH), 7.51-8.36 (m, 9H, aromatic+pyrazole C-5 H), 9.61 (s, 1H, CH=N), 9.94 (s, 1H, OH); MS, m/z (rel. abund. %): 369 (15.1), 367 (42.3), 324 (100), 216 (24.4), 166 (46.2), 108 (87.2), 77 (46.2).

4-{[3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-ylmethylene]amino}benzenesulfonamide (9)

Yield 55%; M.p. > 300°C; Calculated for $C_{22}H_{16}ClN_5O_4S$. 3/4 H_2O (495.43): 53.34 %C, 3.56 %H. Found: 53.33 %C, 3.65 %H; IR, ν cm^{-1}: 3400, 3350 (NH_2), 1600 (C=N); ^{1}H-NMR δ: 6.77-8.35 (m, 15H, aromatic+pyrazole C-5 H+NH_2, the signal at 8.35 was D_2O exchangeable), 9.20 (s, 1H, CH=N); MS, m/z (rel. abund. %): 329 (41.2), 327 (100), 292 (60.7), 246 (25.2), 205 (14.8), 149 (20.2), 111(32.3), 75 (96.5).

N-Acetyl-4-{[3-(4-chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-ylmethylene]amino}benzenesulfonamide (10)

Yield 60%; M.p. > 300°C; Calculated for $C_{24}H_{18}ClN_5O_5S$ (523.95): 55.02 %C, 3.46 %H, 13.37 %N. Found: 54.61 %C, 3.68 %H, 13.12 %N; IR, ν cm^{-1}: 3250 (NH), 1690 (C=O), 1610 (C=N); ^{1}H-NMR δ: 2.47 (s, 3H, CH_3), 6.87-8.23 (m, 13H, aromatic+pyrazole C-5 H), 9.20 (s, 1H, CH=N), 9.99 (s, 1H, NH, D_2O exchangeable); MS, m/z (rel. abund. %): 464 (39.8), 462 (100), 325 (14.4), 279 (26.2), 243 (10.4), 136 (15.9), 64 (33.4).

3-(4-Chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde N-benzylthiosemicarbazone (11)

Yield 65%; M.p.204-206°C; Calculated for $C_{24}H_{20}ClN_5S$ (445.97): 64.64 %C, 4.52 %H, 15.70 %N. Found: 64.35 %C, 4.50 %H, 15.54 %N; IR, ν cm^{-1}: 3300 (NH), 1600 (C=N); ^{1}H-NMR δ: 4.82 (d, J = 6.2 Hz, 2H, CH_2), 7.20-8.72 (m, 15H, aromatic+pyrazole C-5 H), 8.72 (s, 1H, NH, D_2O exchangeable), 8.99 (s, 1H, CH=N), 11.46 (s, 1H, NH, D_2O exchangeable); MS, m/z (rel. abund. %): 447 (0.6, M^++2), 445 (4.3, M^+), 370 (12.0), 338 (26.8), 280 (73.2), 106 (56.7), 77 (100), 51 (70.8).

3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazole-4-carbaldehydeN-benzylthiosemicarbazone (12)

Yield 60%; M.p. > 300°C; Calculated for $C_{24}H_{19}ClN_6O_2S$ (490.97): 58.71 %C, 3.90 %H, 17.12 %N. Found: 58.22 %C, 3.87 %H, 17.41 %N; IR, ν cm^{-1}: 3300 (NH), 1600 (C=N); ^{1}H-NMR δ: 4.89 (d, J = 6.2 Hz, 2H, CH_2), 7.30-8.75 (m, 14H, aromatic+pyrazole C-5 H), 8.75 (s, 1H, NH, D_2O exchangeable), 9.03 (s, 1H, CH=N), 11.48 (s, 1H, NH, D_2O exchangeable).

N'-[3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene-2-hydroxybenzohydrazide (13)

Yield 55%; M.p. 214-216°C; Calculated for $C_{23}H_{17}ClN_4O_2$ (416.86): 66.27 %C, 4.11 %H, 13.44 %N. Found: 66.58 %C, 3.87 %H, 13.80 %N; IR, ν cm^{-1}: 3400 (OH) ,3250 (NH), 1660 (C=O), 1600 (C=N); ^{1}H-NMR δ: 6.97-7.61 (m, 14H, aromatic+pyrazole C-5 H), 9.35 (s, 1H, CH=N), 9.94 (s, 1H, NH, D_2O exchangeable).

N'-[3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-yl]methylene-2-hydroxybenzohydrazide (14)

Yield 52%; M.p. > 300°C; Calculated for $C_{23}H_{16}ClN_5O_4$ (461.86): 59.81%C, 3.49 %H, 15.16 %N. Found: 60.12 %C,

3.12 %H, 14.78 %N; IR, ν cm^{-1}: 3400 (OH) ,3250 (NH), 1660 (C=O), 1600 (C=N); ^{1}H-NMR δ: 7.52-8.31 (m, 13H, aromatic+pyrazole C-5 H), 8.92 (s, 1H, CH=N), 9.85 (s, 1H, NH), 12.18 (s, 1H, OH); MS, m/z (rel. abund. %): 463 (5.8, M^++2), 461 (13.8, M^+), 332 (100), 194 (15.1), 142 (13.5), 110 (62.3).

N'-{[3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene}isonicotinohydrazide (15)

Yield 56%; M.p. 210-212°C; Calculated for $C_{22}H_{16}ClN_5O$. 2 H_2O (437.88): 60.35 %C, 4.60 %H. Found: 60.71 %C, 4.28 %H; IR, ν cm^{-1}: 3200 (NH), 1670 (C=O), 1600 (C=N); ^{1}H-NMR δ: 7.37-8.96 (m, 14H, aromatic+pyrazole C-5 H), 9.34 (s, 1H, CH=N), 9.92 (s, 1H, NH, D_2O exchangeable); MS, m/z (rel. abund. %): 403, (6.2, M^++2), 401 (15, M^+), 279 (100), 106 (32.8), 77 (83.1).

N'-{[3-(4-Chlorophenyl)-1-(4-nitrophenyl)-1H-pyrazol-4-yl]methylene}isonicotinohydrazide (16)

Yield 60%; M.p. > 300°C; Calculated for $C_{22}H_{15}ClN_6O_3$ (446.85): 59.13 %C, 3.38 %H, 18.81 %N. Found: 58.89 %C, 3.25 %H, 19.11 %N; IR, ν cm^{-1}: 3250 (NH), 1668 (C=O), 1600 (C=N); ^{1}H-NMR δ: 7.57-8.90 (m, 13H, aromatic+pyrazole C-5 H), 9.36 (s, 1H, CH=N), 9.95 (s, 1H, NH, D_2O exchangeable).

Preparation of 4-{N'-[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene]hydrazino}benzenesulfonamide (17)

A mixture of **3** (0.01 mole), 4-hydrazinobenzenesulfonamide hydrochloride (2.7 g, 0.012 mole), and sodium acetate (0.8 g, 0.01 mole) in ethanol (20 ml) was refluxed for 6 h, then cooled and poured onto cold water. The precipitated product was filtered, dried and crystallized from ethanol. Yield 70%; M.p. 224-226°C; Calculated for $C_{22}H_{18}ClN_5O_2S$. 1.5 H_2O (478.96): 55.17 %C, 4.42 %H, 14.62 %N. Found: 55.47 %C, 4.31 %H, 14.66 %N; IR, ν cm^{-1}: 3400-3200 (NH_2, NH), 1600 (C=N); ^{1}H-NMR δ: 7.04-7.99 (m, 16H, aromatic+pyrazole C-5 H+ NH_2, the signal at 7..99 was D_2O exchangeable), 8.94 (s, 1H, CH=N), 10.66 (s, 1H, NH, D_2O exchangeable), MS, m/z (rel. abund. %): 453 (29.7, M^++2), 451(87.1, M^+), 280 (34.7), 255 (34.2), 91(44.2), 77(100), 64 (50.5).

Carrageenan-Air Pouch Model

Materials and Methods

An air pouch was induced by subcutaneous injection of 20 ml of air on the back of the rat on day-one. Two days later, another 10 ml of air was injected at the same site. On the fifth day after the first injection, a further 10 ml of air was injected into the pouch. Twenty-four hours later, carrageenan (2 ml of a 1% w/v solution in sterile saline) was injected into the air pouch. All of the injections were performed after the rats had been anaesthetized with ether. Six hours after the carrageenan injection, the rats were anaesthetized with ether and the pouch was carefully opened by a small incision. The exudate was collected and transferred to a sterile tube. The volume of the exudate was measured. Aliquots were diluted to 1:1 with 0.01% (w/v) methylene blue in phosphate-buffered saline- pH 7.2 (PBS), and cells were counted in a standard hematocytometer chamber (American Optical) for characterization of the cellular infiltrate. In one group of rats (n=6), sterile saline (0.9% w/ v; 2 ml) was injected into the air pouch instead of carrageenan.

Test Drugs

One hour prior to injection of carrageenan into the air pouch, the rats (n=6 per group) were orally pretreated with vehicle (1% carboxymethylcellulose) or one of the test drugs at a dose of 10 mg/kg. Another series of experiments were performed, in which the rats received Indomethacin (10 mg/kg; n=6) 1 h prior to injection of carrageenan into the air pouch.

Statistical Analysis

All data are expressed as the mean ± SD. Comparisons of tested drugs versus Carrageenan-treated group were made by unpaired student t-test.

Modeling Studies

Computer-assisted simulated docking experiments were carried out under an MMFF94X force field in Cox-2 structure (PDB ID: 1CX2) using Chemical Computing Group's Molecular Operating Environment (MOE-dock 2006) software, Montréal, Canada.

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